

Minerals of the Radular Apparatus of *Falcidens* sp. (Caudofoveata) and the Evolutionary Implications for the Phylum Mollusca

RENATO CRUZ^{1,*}, ULYSSES LINS², AND MARCOS FARINA^{3,†}

¹*Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Cidade Universitária, 21949-900, Rio de Janeiro, RJ, Brasil;* ²*Instituto de Microbiologia Professor Paulo de Góes, Setor de Microscopia Eletrônica e Departamento de Microbiologia Geral, Universidade Federal do Rio de Janeiro, Cidade Universitária, 21941-590, Rio de Janeiro, RJ, Brasil;* and

³*Departamento de Anatomia, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro, RJ, Brasil*

Abstract. Minerals have been found in the radular teeth of molluscs from the classes Caudofoveata, Polyplacophora, Monoplacophora, and Gastropoda (Patellogastropoda: Acmaeidae, Patellidae). Here we report the discovery of amorphous iron oxide and hydroxyapatite in the highly modified radular apparatus of *Falcidens* sp. (Caudofoveata). The mineralization process in *Falcidens* sp. is unique: the components of the radular apparatus, unlike those of other molluscs, are not renewed during the animal's lifetime. We propose that the presence of mineralized teeth among the molluscs is not necessarily connected to their manner of obtaining food and suggest that the molluscan common ancestor had mineralized teeth.

Introduction

The radula is one of the most characteristic organs of the phylum Mollusca, lacking only in the class Bivalvia. The function of the radula is to obtain food. The basic pattern of most radulae consists of several to numerous transverse rows of radular teeth with bilateral symmetry relative to the main axis, supported by a thin radular membrane. The radula lies within the radular sac, an inva-

gination of the buccal cavity, where it is continuously produced at the proximal end (Brusca and Brusca, 1990). In some molluscan groups, mineralized compounds are associated with the organic matrix of the teeth. Best known among the groups that have mineralized teeth are the limpets (Gastropoda: Acmaeidae, Patellidae) and chitons (Polyplacophora). In chitons, the second lateral tooth is mineralized primarily by magnetite (Fe_3O_4) (Lowenstam, 1962a). However, ferrihydrite ($5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$), lepidocrocite ($\gamma\text{-FeOOH}$), maghemite ($\gamma\text{-Fe}_2\text{O}_3$), goethite ($\alpha\text{-FeOOH}$), and apatite (crystalline calcium phosphate) also occur (Lowenstam, 1967; Towe and Lowenstam, 1967; Kim *et al.*, 1989; St. Pierre *et al.*, 1992). In limpets, the radular teeth are impregnated only with goethite and opal ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Lowenstam, 1962b, 1971).

The Caudofoveata (=Aplacophora; Chaetodermomorpha) is a class of marine worm-like molluscs. Until recently the class had received little attention (Scheltema *et al.*, 1994; Ivanov, 1996) and the only known mineralized structures were the spicules that cover the body. These spicules present different morphologies in different regions of the body surface and are composed of the crystalline calcium carbonate polymorph aragonite (CaCO_3). On the other hand, precipitates rich in aluminum, calcium, phosphorus, and iron have been reported in the teeth of *Chevroderma turnerae* (Prochaetodermatidae) (Tillier and Cuif, 1986). In its jaws, sodium, magnesium, aluminum, phosphorus, calcium, and small quantities of iron have been detected (Tillier and Cuif, 1986). Another class

Received 5 March 1997; accepted 22 December 1997.

* Present address: Universidade Santa Úrsula, Rua Jornalista Orlando Dantas 59, 22231-010, Rio de Janeiro, RJ, Brasil.

† To whom correspondence should be addressed. E-mail: mfarina@ibccf.biof.ufrj.br

only recently studied which has mineralized compounds in its teeth is the Monoplacophora (= Tryblidiida) (iron oxide, Lindberg, 1986; Haszprunar, 1988; McLean, 1990). These results suggest that the more primitive molluscan classes (*e.g.*, Caudofoveata and Monoplacophora) should be investigated as potential models for the study of biomineralization. In the present study, we report the discovery, through analytical electron microscopy, of mineralized compounds in the radular apparatus of *Falcidens* sp. (Caudofoveata:Chaetodermatidae), and we propose some interpretations of these results from an evolutionary perspective.

Materials and Methods

The specimens of *Falcidens* sp. (Rios, 1994) were collected in 1986 on the continental slope (22° 55' S, 42° 00' W and 23° 05' S, 42° 20' W) off Rio de Janeiro State, Brazil, at depths between 25 and 97 m. They were immediately fixed in buffered formaldehyde at 4% for 24 h and stored in 70% ethanol.

Scanning electron microscopy (SEM)

Radular apparatuses were placed under a light microscope and dissected. They were then cleaned in 1 N NaOH for 2 h at 70°C, rinsed in distilled water, dehydrated in an ethanol series, critical-point dried, and gold sputtered. Finally, they were examined with a Zeiss DSM 940.

Transmission electron microscopy (TEM)

Radular apparatuses were viewed under a light microscope and extracted, then embedded in Spurr resin. Ultrathin (≈ 90 – 100 nm) sections were mounted on copper grids and examined without any stain. Transmission electron micrographs and electron spectroscopic diffraction (ESD) patterns (Reimer *et al.*, 1990; Barckhauss *et al.*, 1991) were obtained with an energy filtering electron microscope (Zeiss CEM 902) operating at 80 kV.

Energy dispersive X-ray analysis (EDXA)

Unstained, ultrathin sections were examined in the TEM mode of a Zeiss EM 912 analytical electron microscope, operating at 100 kV and equipped with a silicon/lithium detector (Oxford). Selected areas down to $0.1 \mu\text{m}$ were analyzed for 100 s.

Electron spectroscopic images (ESI)

Unstained, ultrathin (≈ 40 – 60 nm) sections were mounted on copper grids. To determine the net element composition of a specific area, electron spectroscopic images (Bauer, 1988) were recorded using the Zeiss CEM

902 operating at 80 kV (energy-selecting slit: 20 eV) and a digital analyzer (Kontron-Zeiss).

Results

The radular apparatus of the family Chaetodermatidae is highly modified when compared to the basic pattern found in most molluscs (Scheltema, 1972, 1976, 1981; Salvini-Plawen, 1988). The first complete light microscopy description of the *Falcidens* radula was made by Scheltema (1972). In *Falcidens* sp. (Fig. 1, A, B, C) the radular apparatus is constituted by one pair of teeth accompanied by a second denticle-like pair. These teeth are attached to the end of a cone-shaped piece (radular cone). Two lateral membranes extend almost one-third the length of the radular cone. In the main region of the radular apparatus are a circular structure known as the axial plate and one pair of bar-shaped apophyses. Transmission electron micrographs of transverse sections of the unstained radular apparatus of *Falcidens* sp. (Fig. 1D) reveal that the axial plate in fact is composed of two parallel, electron-dense plates. These plates are connected to the radular lateral membrane near the radular cone (Fig. 1E).

EDXA analysis of sections containing the axial plate shows deposits rich in iron, silicon, oxygen, sulfur, phosphorus, and calcium (Fig. 2A). The denticles (Fig. 2B) and the second denticle-like pair also contain these elements. The radular cone, the periphery of the axial plate, and the periphery of the denticles and denticle-like pair do not exhibit mineralized compounds. Frontal sections of a denticle (Fig. 3A) show that the entire tooth is electron dense, except at the periphery, and in some internal regions dark precipitates are observed. When viewed at higher magnification, the dark precipitates are seen as several needlelike crystals (Fig. 3B) oriented parallel to the organic fibers. ESI of iron in sections from the sample in Figure 3A show that this element occurs mainly in the core of the denticle (Fig. 3C). Oxygen was found to be distributed in the whole section of the denticle (Fig. 3D). By the same technique, phosphorus (Fig. 3E) and calcium (Fig. 3F) were shown to be restricted to the periphery, where the precipitates are more electron dense. The association of iron with oxygen is interpreted as iron oxide in an amorphous state, since no diffraction spots were detected by ESD. Diffraction patterns of the regions rich in calcium, phosphorus, and also oxygen are compatible with hydroxyapatite, $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ (Fig. 4).

Discussion

The morphology and the mineralization process in the radular apparatus of *Falcidens* sp. are markedly different from those of chitons and limpets. The radulae of those two groups have several rows of teeth in different stages

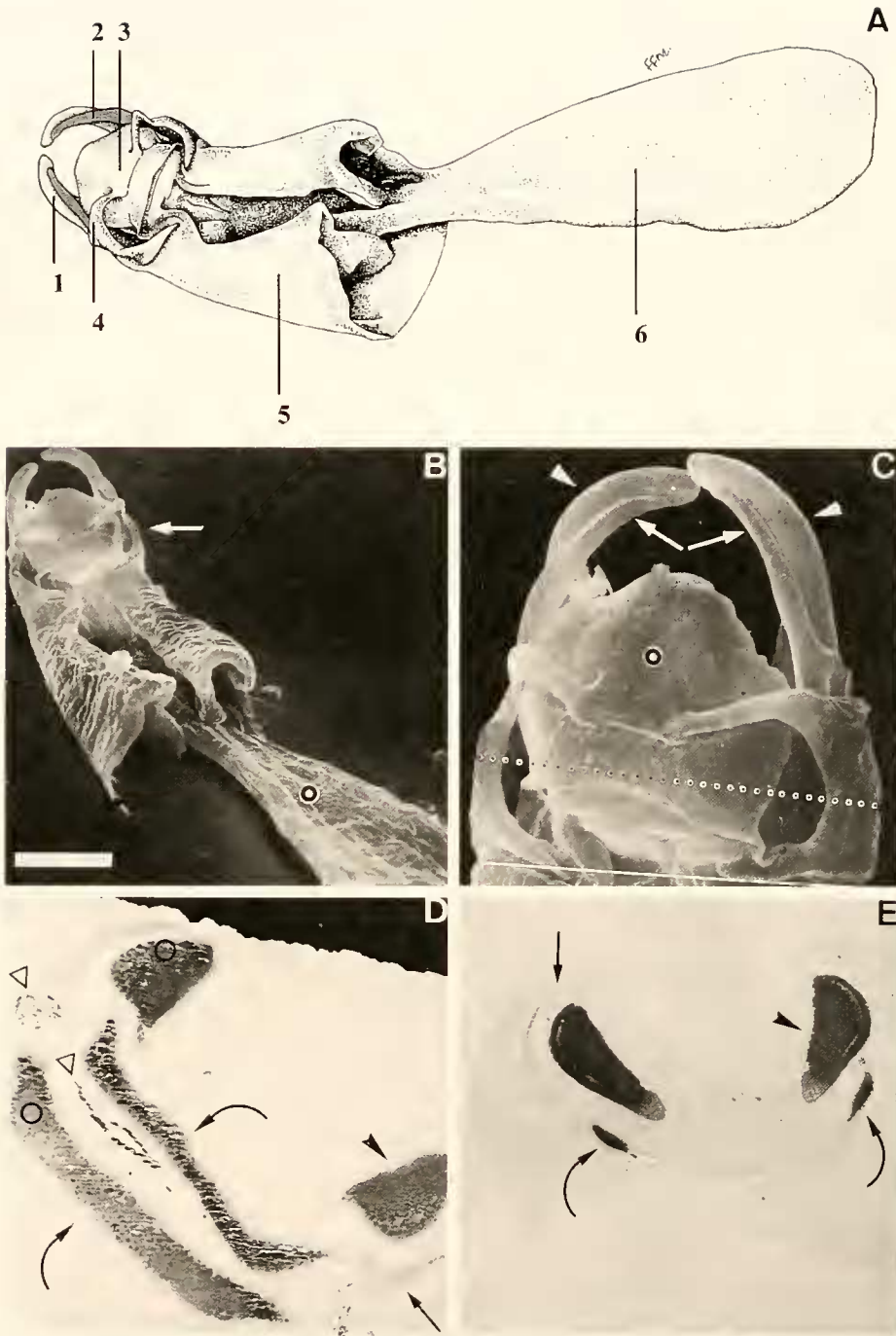


Figure 1. The radular apparatus of *Falcidens* sp. (A) Representative drawing of the apparatus showing denticles (1), second denticle-like pair (2), axial plate (3), bar-shaped apophyses (4), lateral membrane (5), and radular cone (6). (B) Scanning electron micrograph showing the radular cone (open circle) and lateral membrane (arrow). Scale bar in (B), 20 μm . (C) Anterior view of radular apparatus showing a denticle pair (arrowheads), second denticle-like pair (arrows), and axial plate (open circle). The scale bar in (B) represents 4.5 μm in (C). (D) Transmission electron micrograph (TEM) of a transverse section through a region indicated in (C) by the dotted line. Curved arrows show the components of the axial plate; also shown are denticles (arrowhead), second denticle-like pair (arrow), and particles of marine sediments (open arrowheads) that have been retained in the radular apparatus. The open circles indicate the regions selected for energy dispersive X-ray analysis. (E) TEM of a transverse section through the region indicated in (C) by the continuous line. Denticle (arrowhead), second denticle-like pair (arrow), and axial plate (curved arrows) are seen. The scale bar in (B) represents 4.9 μm in (D) and (E).

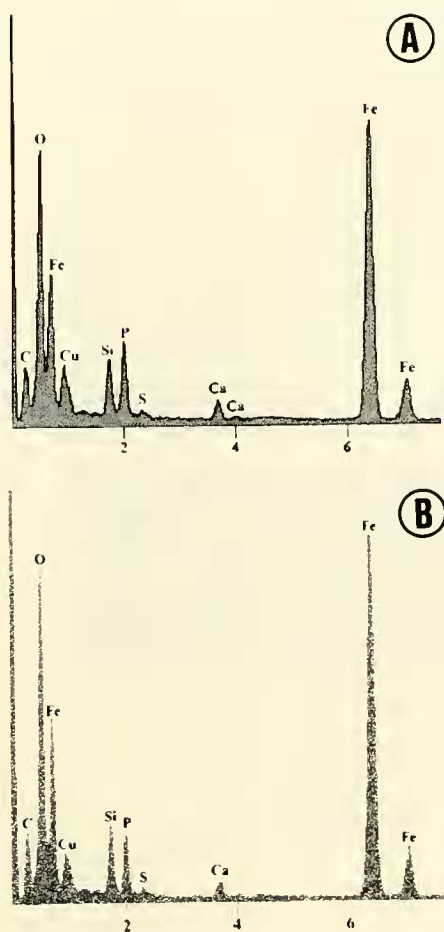


Figure 2. Energy dispersive X-ray analysis spectrum representing the elemental composition of the axial plate (A) and denticle (B) of *Falcidens* sp. The same elements are present in both structures. The x axis represents X-ray energies in kiloelectron volts. The y axis represents counts per second (arbitrary scale for qualitative comparison). The copper peak comes from the grid bar.

of mineralization, ranging from immature teeth (containing only soft organic structures) to fully mineralized mature teeth (Kirschvink and Lowenstam, 1979; Lowenstam and Weiner, 1985; Mann *et al.*, 1986). Most of these animals are herbivorous scrapers (Steneck and Watling, 1982) whose teeth are abraded and broken during feeding (Webb *et al.*, 1989) and are continuously replaced (Webb *et al.*, 1990). The Chaetodermatidae, however, are selective carnivores (Scheltema, 1981), and their denticles, axial plate, and lateral membrane persist throughout their life span (Luitfried von Salvini-Plawen, pers. comm.). Since these structures are not renewed, it seems that a continuous process of mineralization does not occur, in contrast to chitons (Kirschvink and Lowenstam, 1979) and limpets (Mann *et al.*, 1986).

Impregnation of the denticles of *Falcidens* sp. with a

crystalline phosphorous mineral, hydroxyapatite, constitutes an unusual example of mineralization in invertebrates. The great majority of invertebrates exhibit calcium phosphates only in the amorphous state (Taylor and Simkiss, 1994). Among the invertebrate structures reported to have crystalline phosphorous minerals (apatite) are the radular teeth of the Polyplacophora (Lowenstam, 1967), the shells of inarticulate brachiopods (LeGeros *et al.*, 1985), and the shell plates of certain barnacles (Lowenstam *et al.*, 1992). The presence of amorphous iron oxide as a final product of mineralization in the denticles and axial plate of *Falcidens* sp. is also unusual and does not occur among chitons and limpets. In those two groups, the final form of the iron biomineral is invariably crystalline, although the presence of soluble silicon in limpets can dramatically retard the process of iron oxide crystallization in some areas of the tooth (Mann *et al.*, 1986). The presence of silicon associated with iron may account for the amorphous state of the iron in the denticles and axial plate of *Falcidens* sp.

Unlike most chitons (Lowenstam, 1962a; Steneck and Watling, 1982) and limpets (Steneck and Watling, 1982; Van der Wal, 1989), which graze on hard rocks in the intertidal and near-tidal regions, *Falcidens* sp. does not show a clear association between the presence of minerals in its teeth and its manner of obtaining food. In *Falcidens* sp., the denticles are thought to act as pincers, grasping the food (*e.g.*, foraminifers) with precision (Scheltema, 1981). In fact, the great majority of Caudofoveata (including the genus *Falcidens*) obtain food without performing or enduring any kind of abrasive action in their radular teeth (Scheltema, 1981, 1988). The presence of mineralized compounds in the teeth of nongrazing species also occurs among the Polyplacophora: some carnivorous species in that class have teeth mineralized by magnetite even though they are not used to scrape the substrate (Lowenstam and Weiner, 1989). The deep-sea chitons (deposit feeders) that consume marine sediments to extract the nutritional contents also have mineralized teeth, although they do not graze (Lowenstam and Weiner, 1989). However, the buccal musculature in these species is greatly reduced in comparison with that of grazing species from shallow water (Menzies *et al.*, 1973). These observations suggest that the presence of mineralized teeth among the molluscs is not necessarily related solely to grazing. The hypothesis that the mineralization could increase the stiffness of the denticles and thus be an advantage also for nongrazing individuals such as *Falcidens* sp. cannot be rejected.

In a recent phylogenetic study of the extant Mollusca (Scheltema, 1993), the existence of two separate evolutionary molluscan lineages was proposed. The Solenogastres (=Neomeniomorpha), Caudofoveata (=Chaetoder-

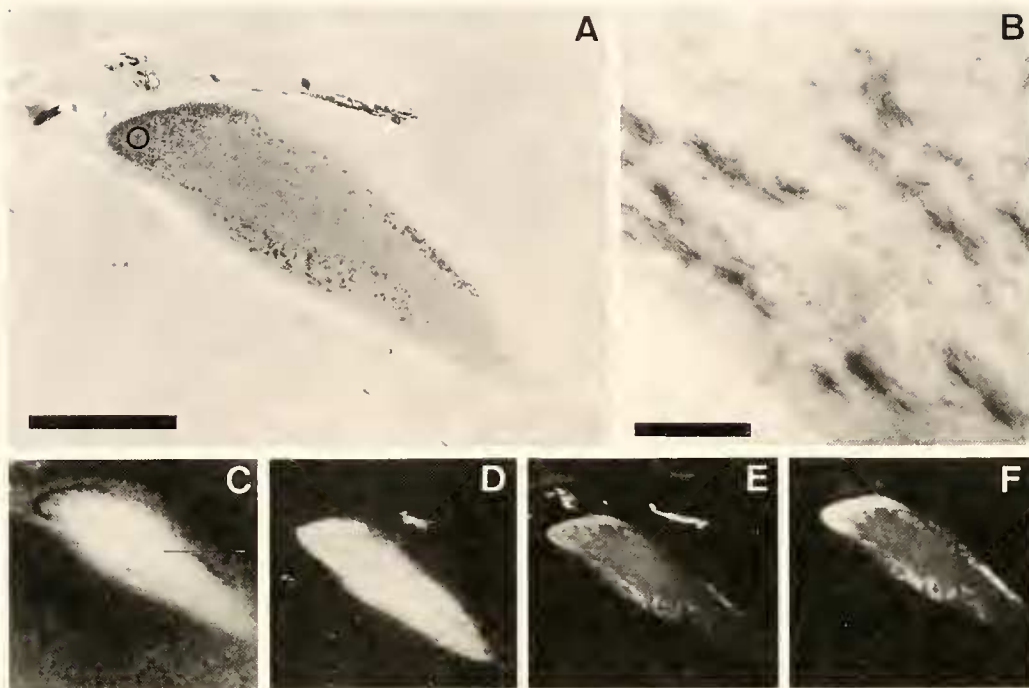


Figure 3. Frontal sections of the denticle of *Falcidens* sp. (A) Transmission electron micrograph of a frontal section showing regions with highly electron-dense precipitates peripheral to less electron-dense region in the core. (B) High magnification of a denticle section showing the presence of several needlelike crystals aligned with the organic fibers. (C–F) Electron spectroscopic images from the section in (A), showing the presence of iron (C), oxygen (D), phosphorus (E), and calcium (F). A comparison of (E) and (F) suggests that the phosphorus is associated with the calcium. Scale bar in (A), 1.25 μm . Scale bar in (B), 90 nm.



Figure 4. Electron spectroscopic diffraction pattern of the dark region (open circle) in Figure 3 A, showing Debye-Scherrer rings of hydroxyapatite. Black arrow indicates hydroxyapatite {002} lattice planes.

momorpha), and Polyplacophora were grouped in the subphylum Aculifera, and the Monoplacophora, Bivalvia, Gastropoda, Scaphopoda, and Cephalopoda were placed in the subphylum Conchifera. The Solenogastres, Caudofoveata, Polyplacophora, and Monoplacophora are considered "primitive" groups (Salvini-Plawen, 1980; Haszprunar, 1988; Scheltema, 1993). Based on the retention of ancestral characters in its radular apparatus (docoglossate radula), the order Patellogastropoda (=Docoglossa)—including the Acmaeidae and Patellidae—is considered to be the earliest offshoot of Gastropoda (Haszprunar, 1988; McLean, 1990). The ancestral characters mentioned above are a stereoglossate radula apparatus with a weakly developed rhachis tooth and mineralized lateral teeth (Haszprunar, 1988). These characters are also present in the Polyplacophora and Monoplacophora (Wingsrand, 1985; Haszprunar, 1988; McLean, 1990). Apart from monoplacophorans and limpets, the Conchifera show no evidence of mineralized compounds in the radulae (Jones *et al.*, 1935; Lowenstam, 1962a; Haszprunar, 1988). We suggest that the presence of mineralized compounds in the radula may be a plesiomorphic character in the phylum Mollusca. The existence of a common molluscan ancestor

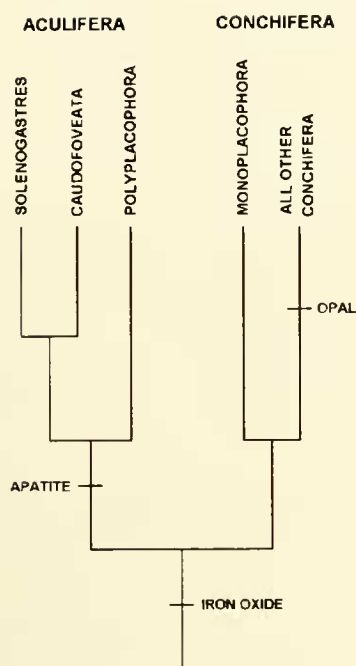


Figure 5. Phylogeny of the extant Mollusca (adapted from Scheltema, 1993). Biogenic minerals in the radular teeth of Mollusca: common molluscan ancestor with mineralized teeth (probably iron oxide); Solenogastres, should be investigated; Caudofoveata, presence of iron oxide and apatite (this work); Polyplacophora, presence of iron oxide and apatite (Lowenstam, 1962a; Lowenstam, 1967); Monoplacophora, presence of iron oxide (Lindberg, 1986); the presence of iron oxide and opal (Lowenstam, 1962b, 1971) was reported only in the families Acmaeidae and Patellidae (Patellogastropoda:Gastropoda). All Other Conchifera refers to the classes Bivalvia, Gastropoda, Scaphopoda, and Cephalopoda. Iron oxide is present in all of the groups that have mineralized teeth.

with mineralized teeth would satisfactorily explain the presence of mineralized compounds in the radulae of different extant molluscan classes (Fig. 5). This hypothesis would be reinforced if mineralized compounds could be found in the teeth of the Solenogastres.

The presence of apatite, a rare mineral among invertebrates, in the same structure (radular teeth) of two distinct groups (Caudofoveata and Polyplacophora) reinforces the evidence that the mineralization of molluscan teeth did not appear independently in these groups, and suggests that this is a heritable character from a common molluscan ancestor.

Acknowledgments

R. C. thanks L. v. Salvini-Plawen for comments and B.Sc. Flávia Moreira Leite for drawing. We thank Laboratório de Biologia Celular e Tecidual (UENF) for EDXA facilities and M. Sorenson for contributions to the final

version of the manuscript. This work was supported by the Brazilian agencies CAPES, FINEP, and CNPq (PRONEX).

Literature Cited

- Barckhaus, R. H., H. J. Höling, I. Fromm, P. Hirsch, and L. Reimer. 1991. Electron spectroscopic diffraction and imaging of the early and mature stages of calcium phosphate formation in the epiphyseal growth plate. *J. Microsc.* **162**: 155–169.
- Bauer, R. 1988. Electron spectroscopic imaging: an advanced technique for imaging and analysis in transmission electron microscopy. *Methods Microbiol.* **20**: 113–146.
- Brusca, R. C., and G. J. Brusca. 1990. *Invertebrates*. Sinauer, Sunderland, MA. 992 pp.
- Haszprunar, G. 1988. On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. *J. Molluscan Stud.* **54**: 367–441.
- Ivanov, D. L. 1996. Origin of Aculifera and problems of monophyly of higher taxa in molluscs. Pp. 59–65 in *Origin and Evolutionary Radiation of the Mollusca*, J. Taylor, ed. London Press, Oxford.
- Jones, E. I., R. A. McCance, and I. R. B. Shackleton. 1935. The role of iron and silica in the structure of the radular teeth of certain marine molluscs. *J. Exp. Biol.* **12**: 59–65.
- Kim, K.-S., D. J. Macey, J. Webb, and S. Mann. 1989. Iron biomineralization in the radula teeth of the chiton *Acanthopleura hirtosa*. *Proc. R. Soc. Lond. Ser. B* **237**: 335–346.
- Kirschvink, J. L., and H. A. Lowenstam. 1979. Mineralization and magnetization of chiton teeth: paleomagnetic, sedimentologic, and biological implications of organic magnetite. *Earth Planet. Sci. Lett.* **44**: 193–204.
- LeGeros, R. Z., C.-M. Pan, S. Suga, and N. Watabe. 1985. Crystallo-chemical properties of apatite in atremate shell. *Calcif. Tissue Int.* **37**: 98–100.
- Lindberg, D. R. 1986. Radula evolution in the Patellogastropoda [Abstract]. *Am. Malacol. Bull.* **4**: 115.
- Lowenstam, H. A. 1962a. Magnetite in denticle capping in recent chitons (Polyplacophora). *Geol. Soc. Am. Bull.* **73**: 435–438.
- Lowenstam, H. A. 1962b. Goethite in radular teeth of recent marine gastropods. *Science* **137**: 279–280.
- Lowenstam, H. A. 1967. Lepidocrocite, an apatite mineral, and magnetite in teeth of chitons (Polyplacophora). *Science* **156**: 1373–1375.
- Lowenstam, H. A. 1971. Opal precipitation by marine gastropods (Mollusca). *Science* **171**: 487–490.
- Lowenstam, H. A., and S. Weiner. 1985. Transformation of amorphous calcium phosphate to crystalline dahlite in the radula teeth of chitons. *Science* **227**: 51–53.
- Lowenstam, H. A., and S. Weiner. 1989. *On Biomineralization*. Oxford University Press, New York. 324 pp.
- Lowenstam, H. A., S. Weiner, and W. A. Newman. 1992. Carbonate apatite-containing shell plates of a barnacle (Cirripedia). Pp. 73–83 in *Chemistry and Biology of Mineralized Tissues*, H. Slavkin and P. Price, eds. Elsevier, Amsterdam.
- Mann, S., C. C. Perry, J. Webb, B. Luke, and R. J. P. Williams. 1986. Structure, morphology, composition and organization of biogenic minerals in limpet teeth. *Proc. R. Soc. Lond. Ser. B* **227**: 179–190.
- McLean, J. H. 1990. Neolepetopsidae, a new docoglossate limpet family from hydrothermal vents and its relevance to patellogastropod evolution. *J. Zool. (Lond.)* **222**: 485–528.
- Menzies, R. J., R. Y. George, and G. T. Rowe. 1973. *Abyssal Environment and Ecology of the World's Oceans*. Wiley-Interscience, New York.

- Reimer, L., I. Fromm, and I. Naundorf. 1990. Electron spectroscopic diffraction. *Ultramicroscopy* **32**: 80–91.
- Rios, E. C. 1994. *Seashells of Brazil*. FURG, Rio Grande do Sul. 368 pp.
- Salvini-Plawen, L. v. 1980. A reconsideration of systematics in the Mollusca (phylogeny and higher classification). *Malacologia* **19**: 249–278.
- Salvini-Plawen, L. v. 1988. The structure and function of molluscan digestive systems. In *The Mollusca*, vol. 11, E. R. Trueman and M. R. Clarke, eds. Academic Press, London.
- Scheltema, A. H. 1972. The radula of the Chaetodermatidae (Mollusca, Aplacophora). *Z. Morphol. Tiere* **72**: 361–370.
- Scheltema, A. H. 1976. Two new species of *Chaetoderma* from off West Africa (Aplacophora, Chaetodermatidae). *J. Molluscan Stud.* **42**: 223–234.
- Scheltema, A. H. 1981. Comparative morphology of the radulae and alimentary tracts in the Aplacophora. *Malacologia* **20**: 361–383.
- Scheltema, A. H. 1988. Ancestors and descendents: relationships of the Aplacophora and Polyplacophora. *Am. Malacol. Bull.* **6**: 57–68.
- Scheltema, A. H. 1993. Aplacophora as progenetic aculiferans and the coelomate origin of mollusks as the sister taxon of Sipunculida. *Biol. Bull.* **184**: 57–78.
- Scheltema, A. H., M. Tscherkassky, and M. Kuzirian. 1994. Aplacophora. In *Microscopic Anatomy of Invertebrates, 5: Mollusca*, F. W. Harrison and A. J. Kohn, eds. John Wiley, New York.
- St. Pierre, T. G., L. A. Evans, and J. Webb. 1992. Non stoichiometric magnetite and maghemite in the mature teeth of the chiton *Acanthopleura hirtosa*. *Hyperfine Interaction* **71**: 1275–1278.
- Steneck, R. S., and L. Watling. 1982. Feeding capabilities and limitations of herbivorous molluscs: a functional group approach. *Mar. Biol.* **68**: 299–319.
- Taylor, M. G., and K. Simkiss. 1994. Cation substitutions in phosphate biominerals. *Bull. Inst. oceanogr. (Monaco) no spécial* **14**: 75–79.
- Tillier, S., and J. P. Cuif. 1986. L'animal-conodonte est-il un mollusque aplacophore? *C. R. Acad. Sci. Paris* **303**: 627–632.
- Towe, K. M., and H. A. Lowenstam. 1967. Ultrastructure and development of iron mineralization in the radula teeth of *Cryptochiton steleri* (Mollusca). *J. Ultrastruct. Res.* **17**: 1–13.
- Van der Wal, P. 1989. Structural and material design of mature mineralized radula teeth of *Patella vulgata* (Gastropoda). *J. Ultrastruct. Mol. Struct. Res.* **102**: 147–161.
- Webb, J., D. J. Macey, and S. Mann. 1989. Biomineralization of iron in molluscan teeth. Pp. 345–387 in *Biomineralization*, S. Mann, J. Webb, and R. J. P. Williams eds. VCH Verlagsgesellschaft, Weinheim.
- Webb, J., T. G. St. Pierre, and D. J. Macey. 1990. Iron biomineralization in invertebrates. Pp. 193–220 in *Iron Biominerals*, R. B. Franke and R. P. Blakemore eds. Plenum Press, New York.
- Wingstrand, K. G. 1985. On the anatomy and relationships of recent Monoplacophora. *Galathea Rep.* **16**: 1–94.